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MEMORANDUM

Date: July 13, 2007

To: Local Health Directors, Hospitals, Private Laboratories

From: Shadia Barghothi, Mycobacteriology Supervisor ^{SSB}
Dr. Samuel Merritt, Microbiology Unit Manager ^{DM}

Cc: Dr. Leslie Wolf, Dr. Lou Turner, Dr. Jeffrey Engel, Vickie Whitaker, Kristy Osterhout

Re: Addition of nucleic acid testing for *Mycobacterium tuberculosis* complex

The North Carolina State Laboratory of Public Health (NCSLPH) will be offering real-time polymerase chain reaction (RT-PCR) testing for *Mycobacterium tuberculosis* (TB) complex on selected specimens beginning 7/23/2007. In many instances RT-PCR test results can provide a more rapid diagnosis of tuberculosis (or exclude such a diagnosis), allowing practitioners to make more informed decisions regarding treatment and the need for isolation. Please note that RT-PCR testing is designed to supplement, rather than to replace, standard mycobacterial culture for confirmation of diagnosis and that the test is not suitable for all specimens. The following disclaimer will appear on all laboratory reports generated by NCSLPH:

****Disclaimer:**

This test is not cleared / approved by the U.S. Food and Drug Administration. It has been validated for use by the NC State Laboratory of Public Health, Mycobacteriology lab, as preliminary identification of *M. tuberculosis* complex from primary patient specimens. Identification should not be based solely on the test results, and must be confirmed by colony morphology.

Real-time PCR will be performed on undigested primary clinical specimens only. The NCSLPH Mycobacteriology laboratory will determine which specimens qualify for testing using the criteria outlined below. It is very important that the Mycobacteriology (TB) requisition form DHHS-1247 is filled out completely, particularly the "Previously Diagnosed", the "Current Condition / Pertinent Data", the "Drug Therapy", and the "Source of Specimen" fields.

1. RT-PCR will be performed on the first AFB smear-positive specimen for each patient.
2. RT-PCR will be performed on smear-negative specimens from patients who are at increased risk of tuberculosis and who demonstrate signs or symptoms consistent with pulmonary TB (See Appendix). No more than three smear-negative specimens will be tested per patient.

Please feel free to contact the Mycobacteriology laboratory at (919) 807-8620 if you have any questions or concerns.



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Guidelines for the Use of Nucleic Acid Amplification Testing for Tuberculosis

Respiratory specimens

RT-PCR can be used on sputum and bronchoalveolar lavage (BAL) samples to rapidly determine whether or not a patient poses an infectious risk to others, particularly in instances where a patient would otherwise be isolated waiting for culture confirmation of a diagnosis (e.g., in a hospital setting). A negative RT-PCR on these samples does not completely exclude the presence of TB; however, a negative test does indicate that the quantity of *M. tuberculosis* in the sputum is a very low level, so the patient is less likely to be contagious. It should not be used in place of sound clinical judgment when determining matters of infectivity or the need for isolation.

Submit three spontaneous or induced sputum specimens (early morning samples are best), or a sample from BAL in addition to a pre- and/or post-BAL specimen. Specimens will be processed as followsⁱ:

1. **AFB smear-positive specimens.** RT-PCR will be performed on the first AFB smear positive specimen for each patient.
 - a. If the RT-PCR result is positive on any AFB smear-positive sample, the patient can be considered to have active TB. RT-PCR will not be performed on subsequent specimens. Specimens will, however, be cultured and TB isolates tested for drug susceptibility as usual.
 - b. If the RT-PCR result is negative on any AFB smear-positive specimen, then testing for inhibitors will be performed.
 - i. If inhibitors are found, the RT-PCR test result is of no diagnostic assistance for that specimen. Up to two more samples can be tested.
 - ii. If no inhibitors are found, testing will continue on other samples. If a second RT-PCR test result on an AFB smear-positive specimen is also negative, the patient can be assumed to have a diagnosis of non-tuberculous mycobacterial disease.
2. **AFB Smear-negative specimens.** Smear-negative specimens will only be tested in patients who are at increased risk of tuberculosis and who demonstrate signs or symptoms consistent with pulmonary TB (See Appendix). Up to three smear-negative specimens will be tested per patient.
 - a. If the RT-PCR test result is positive on any AFB smear-negative specimen, up to two more specimens will be tested. If another specimen also tests positive by RT-PCR, then the patient can be considered to have active TB.
 - b. If the RT-PCR test result is negative on all three AFB smear-negative specimens, the patient can be assumed to be not contagious, and likely does not have TB, though the AFB culture is the final definitive result.
 - c. If the RT-PCR test result is positive on only one specimen, the result is considered indeterminate, and clinical judgment must be used to determine the need for treatment, isolation, and further diagnostic workup.

Extra-pulmonary specimens

Selected specimens from extra-pulmonary sites can be submitted for RT-PCR testing. Specimens appropriate for testing include lymph node aspirate or biopsy, tissue biopsy, and urineⁱⁱ. RT-PCR has a **high specificity on smear-positive specimens** and can confirm a diagnosis of active TB in such instances. However, the sensitivity is much lower, especially on smear-negative specimens, so the RT-PCR test result **cannot be used to exclude a diagnosis of active TB if the specimen is AFB smear-negativeⁱⁱⁱ**. Therefore, only smear-positive tissue specimens will be tested using RT-PCR.

Pleural fluid and cerebrospinal fluid (CSF) are generally not appropriate specimens for RT-PCR testing, as these specimens are generally smear-negative and RT-PCR has very low sensitivity^{iv,v}. Smear-positive pleural fluid or CSF could be submitted for testing, however.

References

- ⁱ. Update: Nucleic acid amplification tests for tuberculosis. *MMWR*. 2000 Jul 7;49(26):593-4.
- ⁱⁱ. Hemal AK, Gupta NP, Rajeev TP, Kumar R, Dar L, Seth P. Polymerase chain reaction in clinically suspected genitourinary tuberculosis: comparison with intravenous urography, bladder biopsy, and urine acid fast bacilli culture. *Urology*. 2000 Oct 1;56(4):570-4.
- ⁱⁱⁱ. Piersimoni C, Scarparo C. Relevance of commercial amplification methods for direct detection of *Mycobacterium tuberculosis* complex in clinical samples. *J Clin Microbiol*. 2003;41:3233-3240.
- ^{iv}. Pai M, Flores LL, Hubbard A, Riley LW, Colford JM Jr. Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis. *BMC Infect Dis*. 2004;4:6.
- ^v. Pai M, Flores LL, Pai N, Hubbard A, Riley LW, Colford JM Jr. Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. *Lancet Infect Dis*. 2003;3:633-643.

Appendix

RT-PCR will be run on AFB smear-negative respiratory specimens only for patients at increased risk of tuberculosis and who demonstrate signs or symptoms compatible with a diagnosis of tuberculosis. If tuberculosis is suspected, indicate on Mycobacteriology (TB) form DHHS-1247 which signs or symptoms are present and which risk factor applies to the patient in the appropriate section. If this information is not supplied, RT-PCR will not be run if the sample is AFB smear-negative. It will still be run if it is smear-positive.

Signs or Symptoms (At least 2 must be present)

Cough
Fever, chills, or night sweats
Significant weight loss
Hemoptysis

Risk factors or other reasons to suspect TB

HIV infection
Cough present for more than 2 weeks
Immigrant from high-incident country
Immunosuppressive medications (includes TNF-alpha inhibitors)
Contact with known TB case in the past 2 years
Leukemia, lymphoma, or cancer of the head and neck or lung
Diabetes mellitus
Silicosis
Gastrectomy or jejunioileal bypass
Injection drug use

Additional criteria to warrant RT-PCR on smear-negative specimen

Please also include the following information on the requisition form (DHHS-1247):

1. Is patient in respiratory isolation?
2. Is patient currently on TB medication? If so, which drugs and for how long?
3. Previously diagnosed
 - a. TB (date: _____)
 - b. Other mycobacterium? Which: _____, when: _____

